



**University of
Zurich^{UZH}**

**Zurich Open Repository and
Archive**

University of Zurich
University Library
Strickhofstrasse 39
CH-8057 Zurich
www.zora.uzh.ch

Year: 2015

Polarized location of SLC and ABC drug transporters in monolayer-cultured human hepatocytes

Le Vee, Marc ; Jouan, Elodie ; Noel, Gregory ; Stieger, Bruno ; Fardel, Olivier

Abstract: Human hepatocytes cultured in a monolayer configuration represent a well-established in vitro model in liver toxicology, notably used in drug transporter studies. Polarized status of drug transporters, i.e., their coordinated location at sinusoidal or canalicular membranes, remains however incompletely documented in these cultured hepatocytes. The present study was therefore designed to analyze transporter expression and location in such cells. Most of drug transporters were first shown to be present at notable mRNA levels in monolayer-cultured human hepatocytes. Cultured human hepatocytes, which morphologically exhibited bile canaliculi-like structures, were next demonstrated, through immunofluorescence staining, to express the influx transporters organic anion transporting polypeptide (OATP) 1B1, OATP2B1 and organic cation transporter (OCT) 1 and the efflux transporter multidrug resistance-associated protein (MRP) 3 at their sinusoidal pole. In addition, the efflux transporters P-glycoprotein and MRP2 were detected at the canalicular pole of monolayer-cultured human hepatocytes. Moreover, canalicular secretion of reference substrates for the efflux transporters bile salt export pump, MRP2 and P-glycoprotein as well as sinusoidal drug transporter activities were observed. This polarized and functional expression of drug transporters in monolayer-cultured human hepatocytes highlights the interest of using this human in vitro cell model in xenobiotic transport studies.

DOI: <https://doi.org/10.1016/j.tiv.2015.03.019>

Posted at the Zurich Open Repository and Archive, University of Zurich

ZORA URL: <https://doi.org/10.5167/uzh-110944>

Journal Article

Accepted Version

Originally published at:

Le Vee, Marc; Jouan, Elodie; Noel, Gregory; Stieger, Bruno; Fardel, Olivier (2015). Polarized location of SLC and ABC drug transporters in monolayer-cultured human hepatocytes. *Toxicology in Vitro*, 29(5):938-946.

DOI: <https://doi.org/10.1016/j.tiv.2015.03.019>

Accepted Manuscript

Polarized location of SLC and ABC drug transporters in monolayer-cultured human hepatocytes

Marc Le Vee, Elodie Jouan, Gregory Noel, Bruno Stieger, Olivier Fardel

PII: S0887-2333(15)00067-3
DOI: <http://dx.doi.org/10.1016/j.tiv.2015.03.019>
Reference: TIV 3499

To appear in: *Toxicology in Vitro*

Received Date: 18 July 2014
Accepted Date: 25 March 2015



Please cite this article as: Le Vee, M., Jouan, E., Noel, G., Stieger, B., Fardel, O., Polarized location of SLC and ABC drug transporters in monolayer-cultured human hepatocytes, *Toxicology in Vitro* (2015), doi: <http://dx.doi.org/10.1016/j.tiv.2015.03.019>

This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Polarized location of SLC and ABC drug transporters in monolayer-cultured human hepatocytes

Marc LE VEE^a, Elodie JOUAN^a, Gregory NOEL^a, Bruno STIEGER^b and Olivier FARDEL^{a,c,*}

^aInstitut de Recherches en Santé, Environnement et Travail (IRSET), UMR INSERM U1085, Faculté de Pharmacie, 2 Avenue du Pr Léon Bernard, 35043 Rennes, France

^bDepartment of Clinical Pharmacology and Toxicology, University Hospital, 8091 Zurich, Switzerland

^cPôle Biologie, Centre Hospitalier Universitaire, 2 rue Henri Le Guilloux, 35033 Rennes, France

* Corresponding author.

Tel : 33 2 23 23 48 90. Fax : 33 2 23 23 47 94. E-mail: olivier.fardel@univ-rennes1.fr

Abstract

Human hepatocytes cultured in a monolayer configuration represent a well-established *in vitro* model in liver toxicology, notably used in drug transporter studies. Polarized status of drug transporters, *i.e.*, their coordinated location at sinusoidal or canalicular membranes, remains however incompletely documented in these cultured hepatocytes. The present study was therefore designed to analyze transporter expression and location in such cells. Most of drug transporters were first shown to be present at notable mRNA levels in monolayer-cultured human hepatocytes. Cultured human hepatocytes, which morphologically exhibited bile canaliculi-like structures, were next demonstrated, through immunofluorescence staining, to express the influx transporters organic anion transporting polypeptide (OATP) 1B1, OATP2B1 and organic cation transporter (OCT) 1 and the efflux transporter multidrug resistance-associated protein (MRP) 3 at their sinusoidal pole. In addition, the efflux transporters P-glycoprotein and MRP2 were detected at the canalicular pole of monolayer-cultured human hepatocytes. Moreover, canalicular secretion of reference substrates for the efflux transporters bile salt export pump, MRP2 and P-glycoprotein as well as sinusoidal drug transporter activities were observed. This polarized and functional expression of drug transporters in monolayer-cultured human hepatocytes highlights the interest of using this human *in vitro* cell model in xenobiotic transport studies.

▶ **Key-words:** Canalicular secretion, Drug transporter, Hepatocytes, Polarization, Primary culture.

1. Introduction

Liver transporters, belonging either to the solute carrier (SLC) transporter family or to the ATP-binding cassette (ABC) transporter family, are now well-recognized as major actors of hepatic drug clearance (Giacomini et al., 2010). Indeed, they are expressed either at the sinusoidal pole of hepatocytes, where they mediate uptake of drugs from blood into hepatocytes, *i.e.*, the so-called phase 0 of the hepatic detoxifying system, or at the canalicular pole of hepatocytes, where they secrete drugs or drug metabolites into the bile, *i.e.*, the so-called phase 3a of the hepatic detoxifying system (Funk, 2008). Additionally, some sinusoidal ABC transporters can secrete drug metabolites back into the blood, *i.e.*, the so-called phase 3b, for a secondary renal elimination (Pfeifer et al., 2014).

Activity of hepatic transporters as well as their expression can be regulated by various drugs (Jigorel et al., 2006; Klaassen and Aleksunes, 2010), which can result in clinically-significant drug-drug interactions through altered hepatic elimination of the co-administrated drugs handled by the targeted transporters (Konig et al., 2013). Other xenobiotics such as environmental pollutants and some physiological factors, including hormones and inflammatory cytokines, can also impair hepatic drug transporter expression and activity (Fardel and Le Vee, 2009; Fardel et al., 2001; Klaassen and Slitt, 2005). Identifying putative interactions of drugs, chemical pollutants or endogenous substances with hepatic drug transporters and characterizing the cellular and molecular mechanisms involved in such interactions are therefore likely important issues that have to be addressed, notably during the development of new molecular entities in pharmaceutical companies, as recently recommended by drug regulatory agencies (Giacomini and Huang, 2013; Prueksaritanont et al., 2013).

For performing such studies on hepatic drug transporter activity and expression, primary cultures of hepatocytes represent an *in vitro* choice model (Brouwer et al., 2013;

Ramboer et al., 2013). For rodent hepatocytes, such cultures are usually performed under a sandwich configuration, *i.e.*, hepatocytes are plated on collagen-coated dishes and overlaid with collagen or matrigel (LeCluyse et al., 1994), which permits to recover a polarized status of hepatocytes and functional bile canaliculi networks (Swift et al., 2010). By contrast, rodent hepatocytes cultivated in a conventional monolayer configuration, *i.e.*, hepatocytes are plated on plastic or collagen-coated dishes, failed to exhibit bile canaliculi-like structures and are therefore not convenient for studying canalicular drug secretion (Luttringer et al., 2002; Noel et al., 2013). In analogy to sandwich-cultured rodent hepatocytes, sandwich-cultured human hepatocytes are thought to represent the gold standard for *in vitro* investigating human hepatic transporters (Bi et al., 2012; De Bruyn et al., 2013). It is however noteworthy that monolayer-cultured human hepatocytes also display notable activity of various drug transporters (Jigorel et al., 2005; Payen et al., 2000) and that absolute quantification of sinusoidal and canalicular transporters through a targeted liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) method revealed no major difference between sandwich- and monolayer-cultured human hepatocytes (Schaefer et al., 2012). Moreover, primary human hepatocytes cultivated in a monolayer configuration retain regulatory ways of transporter expression and have been consequently used for analyzing the effects of drugs or physiological effectors such as cytokines or growth factors on drug transporter levels and activities (Jigorel et al., 2006; Le Vee et al., 2008; Le Vee et al., 2009a; Richert et al., 2009). Whether such monolayer-cultured human hepatocytes exhibit polarized expression of drug transporters, and therefore may closely mimic the *in vivo* situation, remains however yet largely unknown, but is rather important to determine in order to fully assess the relevance of monolayer-cultured human hepatocytes as a valuable *in vitro* model to investigate hepatic drug transport. The present study was therefore designed to get insights about this point, using a combined experimental approach, based on mRNA quantification, immunofluorescence labeling and canalicular

transport assays, and very similar to that recently used for characterizing drug transporter location in highly differentiated human hepatoma HepaRG cells (Le Vee et al., 2013).

2. Materials and methods

2.1 Chemicals

Rhodamine 123, verapamil and probenecid were purchased from Sigma-Aldrich (Saint-Quentin Fallavier, France), whereas carboxy-2,7-dichlorofluoresceine (CF) diacetate was provided by Invitrogen/Life Technologies (Villebon sur Yvette, France). [$^3\text{H}(\text{G})$] taurocholic acid (sp. act. 1.19 Ci/mmol), [6,7- $^3\text{H}(\text{N})$] estrone-3-sulfate (E3S) (sp. act. 57.3 Ci/mmol) and [1- ^{14}C] tetra-ethylammonium (TEA) (sp. act. 2.4 mCi/mmol) were from Perkin-Elmer (Boston, MA). All other chemicals were commercial products of the highest purity available.

2.2 Cell culture

Human hepatocytes were obtained from adult donors undergoing hepatic resection for primary and secondary tumors or other pathologies, via the Biological Resource Center (University Hospital, Rennes, France). Cells were prepared by enzymatic dissociation of histologically-normal liver fragments (Fardel et al., 1993). These freshly isolated hepatocytes were either immediately collected for further RNA isolation and analysis or were seeded on standard tissue-culture plastic plates (BD Biosciences, Le Pont de Claix, France) at a density of approximately 2×10^5 cells/cm² in Williams' E medium (Invitrogen, Cergy-Pontoise, France), supplemented with 10% fetal calf serum (Perbio Sciences, Brébieres, France), 5 µg/ml bovine insulin (Sigma-Aldrich), 100 IU/ml penicillin, 100 µg/ml streptomycin, and 2 mM glutamine (Invitrogen). After 24 h, this seeding medium was discarded, and hepatocytes

were cultured in the fetal calf serum-containing Williams' E medium defined above and supplemented with 5×10^{-5} M hydrocortisone hemisuccinate (Upjohn, Paris La Défense, France) and 2% dimethyl sulfoxide (DMSO), as reported previously (Chouteau et al., 2001; Le Vee et al., 2009b), knowing that DMSO is well-known to promote differentiation and survival of hepatocytes (Isom et al., 1985). The culture medium was routinely renewed every 2 days. All experimental procedures complied with French laws and regulations and were approved by the National Ethics Committee. Hepatocytes usually form nearly-confluent monolayers of viable cells and were used for experiments after a 8-10 days culture period.

2.3 RNA isolation and analysis

Total RNA was isolated from freshly isolated and monolayer-cultured human hepatocytes using the TRIzol reagent (Invitrogen/Life Technologies). RNA (20 ng) was then subjected to reverse transcription-quantitative polymerase chain reaction (RT-qPCR), using the fluorescent dye SYBR Green methodology and an ABI Prism 7300 detector (Applied Biosystem, Foster City, CA, USA), as previously described (Le Vee et al., 2013). The gene primers used in the study were exactly as previously reported (Le Vee et al., 2013; Moreau et al., 2011); their adequate efficiency was routinely checked using serially diluted RNA samples. Amplification curves of the PCR products were analyzed with the ABI Prism SDS software using the comparative cycle threshold (Ct) method. Relative quantification of the steady-state target mRNA levels for transporters was next calculated after normalization of the total amount of cDNA tested to an 18S RNA endogenous reference, knowing that the amount of 18S RNA in freshly isolated human and monolayer-cultured human hepatocytes was found to be constant, *i.e.*, the Ct numbers determined from qPCR assays were similar in these cells (Fig. S1), and was arbitrarily set at 10^6 units in each PCR sample (Moreau et al.,

2011). This finally allowed to get a relative value of expression for each transporter comparatively to the 18S RNA reference.

2.4 Light microscopy analysis

Light microscopy analysis of monolayer cultures of human hepatocytes was performed using an Axiovert microscope (Carl Zeiss, Le Pecq, France), as previously described (Le Vee et al., 2013).

2.5 Immunolocalization studies

Immunofluorescence analyses were performed as previously reported (Le Vee et al., 2009b; Le Vee et al., 2013). Human hepatocytes, cultured on glass coverslips (Millicell EZ slides, Merck Millipore, Billerica, MA) for 8-10 days at the density of approximately 2×10^5 cells/cm² in the medium described above, were first fixed in ice-cold acetone for 10 min. Cells were next incubated for 3 h with mouse monoclonal or rabbit polyclonal antibodies diluted to 1:50 (mouse antibodies) or 1:25 (rabbit antibodies) in phosphate-buffered saline supplemented with 4 % (weight/weight) bovine serum albumin. The mouse monoclonal antibodies were directed against organic cation transporter 1 (OCT1)/SLC22A1 (Abcam, Cambridge, UK), multidrug resistance gene 1 (MDR1)/ABCB1/P-glycoprotein (Alexis Corporation, Lausen, Switzerland), multidrug drug resistance-associated protein (MRP) 2/ABCC2 or MRP3/ABCC3 (Merck Millipore), whereas the rabbit polyclonal antibodies were raised against organic anion transporting polypeptide (OATP) 1B1/SLCO1B1 or OATP2B1/SLCO2B1 (Huber et al., 2007). Controls were performed in parallel with appropriate mouse or rabbit isotypic Ig controls. After washing, goat AlexaFluor 488-labeled secondary antibodies (Invitrogen/Life Technologies) were added for 1 h, and nuclei were subsequently stained with 4,6-diamidino-2-phenylindole. Immunofluorescence images were

finally detected with a Zeiss Axioskop A1 microscope (Carl Zeiss) and a Nikon DS-2MBW camera (Nikon, Champigny-sur-Marne, France), using the NIS-Elements F3.2 software (Nikon).

2.6 Drug transport assays

2.6.1 Sinusoidal influx assay

Uptake transport activities due to the sinusoidal transporters sodium-taurocholate cotransporting polypeptide (NTCP/SLC10A1), OATPs and OCT1 were analyzed through measuring sodium-dependent-intracellular accumulation of the NTCP substrate taurocholate, probenecid-sensitive uptake of the OATP substrate E3S and verapamil-sensitive uptake of the OCT1 substrate TEA, using a defined transport assay medium consisting of 5.3 mM KCl, 1.1 mM KH₂PO₄, 0.8 mM MgSO₄, 1.8 mM CaCl₂, 11 mM d-glucose, 10 mM HEPES, and 136 mM N-methyl glucamine (sodium-free buffer) or 136 mM NaCl (standard sodium-containing buffer), as previously described (Noel et al., 2013). Briefly, cells were incubated at 37 °C for 10 min in the transport medium supplemented with 43.4 nM [³H] taurocholate in the presence or absence of sodium, 3.4 nM [³H] E3S in the presence or absence of the OATP inhibitor probenecid (2 mM) or 40 μM [¹⁴C] TEA in the presence or absence of the OCT1 inhibitor verapamil (50 μM). After washing in phosphate-buffered saline, cells were lysed and accumulation of radiolabeled substrates was determined through scintillation counting. Taurocholate accumulation values in the presence of sodium minus accumulation values in the absence of sodium, E3S uptake values in the absence of probenecid minus uptake values in the presence of probenecid and TEA uptake values in the absence of verapamil minus uptake values in the presence of verapamil are thought to represent NTCP-, OATPs- and OCT1-related transport activities (Jigorel et al., 2005).

2.6.2 Canalicular secretion assay

Biliary secretion of taurocholate, CF and rhodamine 123 was determined through measurement of radiolabeled or fluorescent substrate accumulation into bile canaliculi-like structures, as previously reported (Annaert et al., 2001; Le Vee et al., 2013). Briefly, monolayer-cultured human hepatocytes were first incubated for 10 min at 37°C with the defined Ca^{2+} -containing transport assay buffer described above or with the same buffer, except that 1.8 mM CaCl_2 was withdrawn and 100 μM EGTA was added; such an incubation in this Ca^{2+} -free buffer is known to promote disruption of tight junctions and opening of bile canaliculi networks. Buffers were then removed and cultured human hepatocytes cells were further incubated for 10 min at 37°C in transport assay medium containing 43.4 nM [^3H] taurocholate, 3 μM CF diacetate or 5.25 μM rhodamine 123. After washing with ice-cold phosphate-buffered saline, accumulations of taurocholate, CF or rhodamine 123 into cells + bile canaliculi (Ca^{2+} -containing conditions) and into cells only (Ca^{2+} -free conditions) were determined by scintillation counting (for taurocholate) or spectrofluorimetry (for CF and rhodamine 123) using a SpectraMax Gemini SX spectrofluorometer (Molecular Devices, Sunnyvale, CA) (excitation and emission wavelengths were 485 and 535 nm, respectively). Biliary excretion index (BEI) was finally calculated using the following equation (Liu et al., 1999a):

$$\text{BEI} = \frac{\text{Accumulation (Cells+Bile canaliculi)} - \text{Accumulation (Cells)}}{\text{Accumulation (Cells+Bile canaliculi)}} \times 100$$

2.7 Statistical analysis. Quantitative data were analyzed using the two-sided Student's t-test.

The criterion of significance was $p < 0.05$.

3. Results

Relative mRNA expressions of SLC and ABC drug transporters in monolayer-cultured human hepatocytes and freshly isolated human hepatocytes were first quantified by RT-qPCR. As shown in Fig. 1, expressions of the sinusoidal SLC transporters NTCP and OCT1 as well as those of the sinusoidal ABC efflux transporters MRP1 and MRP3 were similar and not statistically different in both cultured human hepatocytes and freshly isolated human hepatocytes. By contrast, mRNA expressions of the sinusoidal SLC transporters OATP1B1, OATP2B1, organic anion transporter 2 (OAT2/SLC22A7) and, in a more marked manner, OATP1B3 (SLCO1B3), were reduced in cultured human hepatocytes when compared to freshly isolated hepatocytes; the repression factors, *i.e.*, the ratio mRNA levels in freshly isolated hepatocytes versus those in primary counterparts, range for these transporters from 1.8 (for OATP2B1) to 22.2 (for OATP1B3). Expressions of the sinusoidal efflux transporters MRP4 (ABCC4), MRP5 (ABCC5) and MRP6 (ABCC6) were found to be induced in monolayer-cultured human hepatocytes (Fig. 1), knowing that MRP4 and MRP5, in contrast to MRP6, remain rather poorly expressed in monolayer-cultured hepatocytes (expression < 1 arbitrary unit for MRP4 and MRP5 versus expression = 177.5 arbitrary units for MRP6). With respect to canalicular transporters (Fig. 2), MDR1 and MRP2 mRNA levels were up-regulated in cultured human hepatocytes when compared to freshly isolated hepatocytes, whereas those of bile salt export pump (BSEP/ABCB11) and breast cancer resistance protein (BCRP/ABCG2) were reduced and those of multidrug and toxin extrusion 1 transporter (MATE1/SLC47A1) unchanged.

To schematically categorize drug transporter expression in freshly isolated human hepatocytes and in monolayer-cultured counterparts, transporters were next classified according to their level of mRNA expression: low expression (<1 unit), moderate expression (1-15 units), high expression (15-100 units) and very high expression (>100 units) (Table 1).

Most of drug transporters were ranged into the same expression level category in both freshly isolated human hepatocytes and monolayer-cultured counterparts (Table 1). In particular, OCT1 exhibited a very high expression, whereas MRP1, MRP4 and MRP5 were among the less expressed.

Light-microscopic analysis of hepatocyte cultures next demonstrated that monolayer-cultured human hepatocytes exhibited refringent bile canaliculi-like structures, allowing to discriminate sinusoidal-like and canalicular-like membranes (Fig. 3A). Immunofluorescence labeling then revealed that various sinusoidal hepatic transporters such as OATP1B1, OATP2B1, OCT1 and MRP3 were expressed at the sinusoidal pole of monolayer-cultured human hepatocytes cells (Fig. 3B). With respect to the canalicular ABC transporters MDR1/P-glycoprotein and MRP2, they were located to the rather wide bile canaliculi-like structures exhibited by cultured hepatocytes (Fig. 3B).

In order to determine whether sinusoidal transporters were active in monolayer-cultured human hepatocytes, activities of NTCP, OATPs and OCT1 were then determined through measuring intracellular accumulation of reference substrates. As shown in Fig. 4, cultured human hepatocytes exhibited sodium-dependent taurocholate accumulation, thus indicating the presence of NTCP activity in these cells. In the same way, probenecid-inhibitable accumulation of the OATP substrate E3S and verapamil-inhibitable accumulation of the OCT1 substrate TEA were clearly detected in cultured human hepatocytes (Fig. 5), thus reflecting OATP and OCT1 activity in such cells.

To finally investigate whether bile canaliculi-like structures were functional in monolayer-cultured human hepatocytes, canalicular secretions of taurocholate, a substrate for BSEP (Stieger, 2011), of rhodamine 123, a substrate for MDR1/P-glycoprotein (Drenou et al., 1993) and of CF, a substrate for MRP2 (Ellis et al., 2014; Laupeze et al., 2001) were analyzed and quantified using the BEI approach based on Ca^{2+} withdrawal-mediated disruption of

canaliculi (Liu et al., 1999b). Cultured human hepatocytes pre-incubated in Ca^{2+} -free conditions exhibited decreased retention of taurocholate, rhodamine 123 and CF, comparatively to counterparts maintained in the presence of Ca^{2+} (Fig. 5). This demonstrates that monolayer-cultured human hepatocytes secreted taurocholate, rhodamine 123 and CF in bile canaliculi-like structures, allowing to calculate BEI values (55.1 %, 22.1 % and 10.9 % for taurocholate, CF and rhodamine 123).

4. Discussion

The data reported in the present study demonstrate that human hepatocytes cultured in a standard monolayer configuration exhibit polarized expression of various sinusoidal and canalicular drug transporters, associated to detectable sinusoidal drug transporter activities and to the presence of functional bile canaliculi-like structures. Such data highlight the relative similarity to the *in vivo* situation of these human hepatocyte cultures and, consequently, their potential interest for hepatic drug transport studies.

This conclusion is also supported by the fact that most of sinusoidal and canalicular transporters were expressed at notable mRNA levels in monolayer-cultured human hepatocytes, even if, for some of them such as OATPs and OAT2, mRNA expression was rather decreased when compared to levels found in freshly isolated hepatocytes. The global expression of main hepatic drug transporters at the mRNA level, associated to detectable activities of NTCP, OATPs and OCT1, was moreover observed in human hepatocytes maintained in primary culture for 8-10 days, which fully agrees with the conclusion that monolayer-cultured human hepatocytes retain notable levels of drug transporter activity and protein expression with time in culture (Jigorel et al., 2005; Schaefer et al., 2012). By contrast, drug transporter expression, especially that of sinusoidal SLC transporters, is rapidly altered during primary culture for rodent hepatocytes, whatever the culture conditions

(Luttringer et al., 2002; Noel et al., 2013; Tchapanian et al., 2011), which has been hypothesized to reflect the *in vitro* acquisition of a cholestatic phenotype (Wilson et al., 2014). NTCP expression, assessed by LC/MS-MS, as well as taurocholate and rosuvastatin uptake are thus reduced in sandwich-cultured rat hepatocytes with culture time, but are well maintained in human counterparts (Kotani et al., 2011; Qiu et al., 2013). This highlights inter-species discrepancies with respect to expression of highly-differentiated hepatocyte markers such as drug transporters in primary hepatocytes. Similarly, expression of other liver-specific markers such as drug-metabolizing cytochromes P-450 has been shown to be better preserved in cultured human hepatocytes (Morel et al., 1990), which is fully consistent with the conclusion that primary human hepatocytes are phenotypically more stable than rodent counterparts (Guillouzo, 1998).

It is however noteworthy that some differences between freshly isolated human hepatocytes and monolayer-cultured counterparts with respect to transporter mRNA expressions exist and have to be kept in mind when using primary human hepatocytes for drug transport studies. It is notably the case for the sinusoidal transporter OATP1B3, whose expression is markedly reduced in monolayer-cultured human hepatocytes. Uptake of OATP1B3 specific substrates such as some digitalis-like compounds (Gozalpour et al., 2014) may therefore be under-estimated when using monolayer-cultured human hepatocytes for investigating kinetic features of hepatobiliary disposition of these compounds. The sinusoidal efflux orphan transporter MRP6, involved in the autosomal recessive metabolic disease pseudoxanthoma elasticum (Pahlman et al., 1984), is, by contrast, markedly up-regulated in monolayer-cultured human hepatocytes compared to freshly isolated counterparts. Transport of drugs putatively handled by MRP6 and that remain to be unambiguously identified, may consequently be over-estimated with the use of primary human hepatocytes. Other ABC transporters such as MDR1, MRP4 and MRP5 were also induced in monolayer-cultured

human hepatocytes, even if they remained among the less expressed drug transporters (Fig. 3); these efflux transporters have similarly been found to be induced in primary mouse hepatocytes and in well-differentiated hepatoma HepaRG cells when compared to freshly isolated hepatocytes (Le Vee et al., 2013; Noel et al., 2013); such up-regulations can therefore be interpreted as a key hall-mark of hepatocytes when placed in an *in vitro* environment.

Primary human hepatocytes used in the present study were cultured in a monolayer configuration, *i.e.*, they were seeded on plastic dishes without subsequent addition of a collagen or matrigel overlayer. Culturing hepatocytes in sandwich configuration, which is thought to represent the gold standard for drug transport studies with hepatocytes (Bi et al., 2012; Swift et al., 2010), seems therefore to be not an absolute requirement for getting polarized hepatocytes, at least for human hepatocytes. This assertion is interestingly supported by the fact that calcium-sensitive efflux of taurocholate has been previously reported in collagen monolayer-cultured human hepatocytes (Kostrubsky et al., 2003), which likely suggests the presence of functional bile canaliculi-like structures in such cells. Moreover, no relevant difference for sinusoidal and canalicular drug transporter protein levels between sandwich- and monolayer-cultured human hepatocytes has been recently demonstrated through LC/MS-MS-based absolute quantification of transporters (Schaefer et al., 2012). Finally, highly-differentiated human hepatoma HepaRG cells exhibit polarized location of main drug transporters associated to taurocholate canalicular secretion, without being cultured in a sandwich configuration (Le Vee et al., 2013; Sharanek et al., 2014). Interestingly, a shared component between the culture medium used for our primary human hepatocytes and that used for HepaRG cells is DMSO, which is well known to favor hepatocyte survival and differentiation (Choi et al., 2009; Isom et al., 1985; Noel et al., 2013) and to increase Ntcp and Oatp activity in primary rat hepatocytes (Jigorel et al., 2005). The way by which DMSO may promote polarized *in vitro* expression of drug transporters remains nevertheless to be

determined. It can be hypothesized that DMSO may induce formation and secretion of extracellular matrix components in primary cultures of hepatocytes, which may mimic the addition of a collagen or matrigel overlay. To clarify the exact role played by DMSO, additional studies comparing cultured human hepatocytes maintained in the presence or absence of this solvent are however likely required. Otherwise, it is noteworthy that monolayer hepatocyte cultures used in the present study were nearly confluent. This parameter has probably to be taken into account owing to the well-established influence of cell density on differentiated functions of cultured hepatocytes (Greuet et al., 1997).

The canalicular transporters BSEP, MRP2 and MDR1/P-glycoprotein were demonstrated to be functional in our primary human hepatocyte cultures, as demonstrated by the secretion of taurocholate, CF and rhodamine 123 into bile canaliculi-like structures, thus allowing to calculate BEI values, in a way similar to that used for sandwich-cultured hepatocytes. The BEI values for taurocholate (BEI=55.1 %) and CF (BEI=22.1 %) in monolayer-cultured human hepatocytes were however substantially lower than those previously reported in sandwich-cultured human counterparts (BEI around 70-75 % and BEI=34.0%, for taurocholate and CF, respectively) (Hoffmaster et al., 2004; Marion et al., 2007), which may indicate higher activity of BSEP and MRP2 in hepatocytes maintained in a three-dimensional configuration. With respect to rhodamine 123, the BEI value found in our human hepatocyte cultures (BEI=10.9 %) was low, knowing however that rather low BEI values (BEI around 10-20 %) have also been reported for rhodamine 123 in sandwich-cultured rat and mouse hepatocytes (Annaert et al., 2001; Noel et al., 2013). Additional studies are likely required to carefully and more extensively compare BEI values for drugs in monolayer- and sandwich-cultured human hepatocytes.

In summary, primary human hepatocytes maintained in a monolayer configuration were found to exhibit a polarized location of various major sinusoidal and canalicular drug

transporters, associated with efficient canalicular secretion of ABC transporter substrates and detectable sinusoidal drug transporter activities. Monolayer-cultured human hepatocytes have otherwise been demonstrated to retain signaling ways involved in transporter regulation (Jigorel et al., 2006). Taken together, these data indicate that primary human hepatocytes cultured in a monolayer dimension may likely represent a valuable *in vitro* model for studying activity and regulation of drug transporters.

References

- Annaert, P.P., Turncliff, R.Z., Booth, C.L., Thakker, D.R., Brouwer, K.L., 2001. P-glycoprotein-mediated in vitro biliary excretion in sandwich-cultured rat hepatocytes. *Drug Metab Dispos* 29, 1277-1283.
- Bi, Y.A., Kimoto, E., Sevidal, S., Jones, H.M., Barton, H.A., Kempshall, S., Whalen, K.M., Zhang, H., Ji, C., Fenner, K.S., El-Kattan, A.F., Lai, Y., 2012. In vitro evaluation of hepatic transporter-mediated clinical drug-drug interactions: hepatocyte model optimization and retrospective investigation. *Drug Metab Dispos* 40, 1085-1092.
- Brouwer, K.L., Keppler, D., Hoffmaster, K.A., Bow, D.A., Cheng, Y., Lai, Y., Palm, J.E., Stieger, B., Evers, R., 2013. In vitro methods to support transporter evaluation in drug discovery and development. *Clin Pharmacol Ther* 94, 95-112.
- Choi, S., Sainz, B., Jr., Corcoran, P., Uprichard, S., Jeong, H., 2009. Characterization of increased drug metabolism activity in dimethyl sulfoxide (DMSO)-treated Huh7 hepatoma cells. *Xenobiotica* 39, 205-217.
- Chouteau, P., Le Seyec, J., Cannie, I., Nassal, M., Guguen-Guillouzo, C., Gripon, P., 2001. A short N-proximal region in the large envelope protein harbors a determinant that contributes to the species specificity of human hepatitis B virus. *J Virol* 75, 11565-11572.
- De Bruyn, T., Chatterjee, S., Fattah, S., Keemink, J., Nicolai, J., Augustijns, P., Annaert, P., 2013. Sandwich-cultured hepatocytes: utility for in vitro exploration of hepatobiliary drug disposition and drug-induced hepatotoxicity. *Expert Opin Drug Metab Toxicol* 9, 589-616.
- Drenou, B., Fardel, O., Amiot, L., Fauchet, R., 1993. Detection of P glycoprotein activity on normal and leukemic CD34+ cells. *Leuk Res* 17, 1031-1035.
- Ellis, L.C., Grant, M.H., Hawksworth, G.M., Weaver, R.J., 2014. Quantification of biliary excretion and sinusoidal excretion of 5(6)-carboxy-2',7'-dichlorofluorescein (CDF) in

- cultured hepatocytes isolated from Sprague Dawley, Wistar and Mrp2-deficient Wistar (TR(-)) rats. *Toxicol In Vitro* 28, 1165-1175.
- Fardel, O., Le Vee, M., 2009. Regulation of human hepatic drug transporter expression by pro-inflammatory cytokines. *Expert Opin Drug Metab Toxicol* 5, 1469-1481.
- Fardel, O., Morel, F., Guillouzo, A., 1993. P-glycoprotein expression in human, mouse, hamster and rat hepatocytes in primary culture. *Carcinogenesis* 14, 781-783.
- Fardel, O., Payen, L., Courtois, A., Vernhet, L., Lecureur, V., 2001. Regulation of biliary drug efflux pump expression by hormones and xenobiotics. *Toxicology* 167, 37-46.
- Funk, C., 2008. The role of hepatic transporters in drug elimination. *Expert Opin Drug Metab Toxicol* 4, 363-379.
- Giacomini, K.M., Huang, S.M., 2013. Transporters in drug development and clinical pharmacology. *Clin Pharmacol Ther* 94, 3-9.
- Giacomini, K.M., Huang, S.M., Tweedie, D.J., Benet, L.Z., Brouwer, K.L., Chu, X., Dahlin, A., Evers, R., Fischer, V., Hillgren, K.M., Hoffmaster, K.A., Ishikawa, T., Keppler, D., Kim, R.B., Lee, C.A., Niemi, M., Polli, J.W., Sugiyama, Y., Swaan, P.W., Ware, J.A., Wright, S.H., Yee, S.W., Zamek-Gliszczynski, M.J., Zhang, L., 2010. Membrane transporters in drug development. *Nat Rev Drug Discov* 9, 215-236.
- Gozalpour, E., Greupink, R., Wortelboer, H.M., Bilos, A., Schreurs, M., Russel, F.G., Koenderink, J.B., 2014. Interaction of Digitalis-Like Compounds with Liver Uptake Transporters NTCP, OATP1B1, and OATP1B3. *Mol Pharm* 11, 1844-1855.
- Greuet, J., Pichard, L., Ourlin, J.C., Bonfils, C., Domergue, J., Le Treut, P., Maurel, P., 1997. Effect of cell density and epidermal growth factor on the inducible expression of CYP3A and CYP1A genes in human hepatocytes in primary culture. *Hepatology* 25, 1166-1175.
- Guillouzo, A., 1998. Liver cell models in in vitro toxicology. *Environ Health Perspect* 106 Suppl 2, 511-532.

- Hoffmaster, K.A., Turncliff, R.Z., LeCluyse, E.L., Kim, R.B., Meier, P.J., Brouwer, K.L., 2004. P-glycoprotein expression, localization, and function in sandwich-cultured primary rat and human hepatocytes: relevance to the hepatobiliary disposition of a model opioid peptide. *Pharm Res* 21, 1294-1302.
- Huber, R.D., Gao, B., Sidler Pfandler, M.A., Zhang-Fu, W., Leuthold, S., Hagenbuch, B., Folkers, G., Meier, P.J., Stieger, B., 2007. Characterization of two splice variants of human organic anion transporting polypeptide 3A1 isolated from human brain. *Am J Physiol Cell Physiol* 292, C795-806.
- Isom, H.C., Secott, T., Georgoff, I., Woodworth, C., Mummaw, J., 1985. Maintenance of differentiated rat hepatocytes in primary culture. *Proc Natl Acad Sci U S A* 82, 3252-3256.
- Jigorel, E., Le Vee, M., Boursier-Neyret, C., Bertrand, M., Fardel, O., 2005. Functional expression of sinusoidal drug transporters in primary human and rat hepatocytes. *Drug Metab Dispos* 33, 1418-1422.
- Jigorel, E., Le Vee, M., Boursier-Neyret, C., Parmentier, Y., Fardel, O., 2006. Differential regulation of sinusoidal and canalicular hepatic drug transporter expression by xenobiotics activating drug-sensing receptors in primary human hepatocytes. *Drug Metab Dispos* 34, 1756-1763.
- Klaassen, C.D., Aleksunes, L.M., 2010. Xenobiotic, bile acid, and cholesterol transporters: function and regulation. *Pharmacol Rev* 62, 1-96.
- Klaassen, C.D., Slitt, A.L., 2005. Regulation of hepatic transporters by xenobiotic receptors. *Curr Drug Metab* 6, 309-328.
- Konig, J., Muller, F., Fromm, M.F., 2013. Transporters and drug-drug interactions: important determinants of drug disposition and effects. *Pharmacol Rev* 65, 944-966.
- Kostrubsky, V.E., Strom, S.C., Hanson, J., Urda, E., Rose, K., Burliegh, J., Zocharski, P., Cai, H., Sinclair, J.F., Sahi, J., 2003. Evaluation of hepatotoxic potential of drugs by inhibition

- of bile-acid transport in cultured primary human hepatocytes and intact rats. *Toxicol Sci* 76, 220-228.
- Kotani, N., Maeda, K., Watanabe, T., Hiramatsu, M., Gong, L.K., Bi, Y.A., Takezawa, T., Kusuhashi, H., Sugiyama, Y., 2011. Culture period-dependent changes in the uptake of transporter substrates in sandwich-cultured rat and human hepatocytes. *Drug Metab Dispos* 39, 1503-1510.
- Laupeze, B., Amiot, L., Payen, L., Drenou, B., Grosset, J.M., Lehne, G., Fauchet, R., Fardel, O., 2001. Multidrug resistance protein (MRP) activity in normal mature leukocytes and CD34-positive hematopoietic cells from peripheral blood. *Life Sci* 68, 1323-1331.
- Le Vee, M., Gripon, P., Stieger, B., Fardel, O., 2008. Down-regulation of organic anion transporter expression in human hepatocytes exposed to the proinflammatory cytokine interleukin 1beta. *Drug Metab Dispos* 36, 217-222.
- Le Vee, M., Lecureur, V., Moreau, A., Stieger, B., Fardel, O., 2009a. Differential regulation of drug transporter expression by hepatocyte growth factor in primary human hepatocytes. *Drug Metab Dispos* 37, 2228-2235.
- Le Vee, M., Lecureur, V., Stieger, B., Fardel, O., 2009b. Regulation of drug transporter expression in human hepatocytes exposed to the proinflammatory cytokines tumor necrosis factor-alpha or interleukin-6. *Drug Metab Dispos* 37, 685-693.
- Le Vee, M., Noel, G., Jouan, E., Stieger, B., Fardel, O., 2013. Polarized expression of drug transporters in differentiated human hepatoma HepaRG cells. *Toxicol In Vitro* 27, 1979-1986.
- LeCluyse, E.L., Audus, K.L., Hochman, J.H., 1994. Formation of extensive canalicular networks by rat hepatocytes cultured in collagen-sandwich configuration. *Am J Physiol* 266, C1764-1774.

- Liu, X., LeCluyse, E.L., Brouwer, K.R., Gan, L.S., Lemasters, J.J., Stieger, B., Meier, P.J., Brouwer, K.L., 1999a. Biliary excretion in primary rat hepatocytes cultured in a collagen-sandwich configuration. *Am J Physiol* 277, G12-21.
- Liu, X., LeCluyse, E.L., Brouwer, K.R., Lightfoot, R.M., Lee, J.I., Brouwer, K.L., 1999b. Use of Ca^{2+} modulation to evaluate biliary excretion in sandwich-cultured rat hepatocytes. *J Pharmacol Exp Ther* 289, 1592-1599.
- Luttringer, O., Theil, F.P., Lave, T., Wernli-Kuratli, K., Guentert, T.W., de Saizieu, A., 2002. Influence of isolation procedure, extracellular matrix and dexamethasone on the regulation of membrane transporters gene expression in rat hepatocytes. *Biochem Pharmacol* 64, 1637-1650.
- Marion, T.L., Leslie, E.M., Brouwer, K.L., 2007. Use of sandwich-cultured hepatocytes to evaluate impaired bile acid transport as a mechanism of drug-induced hepatotoxicity. *Mol Pharm* 4, 911-918.
- Moreau, A., Le Vee, M., Jouan, E., Parmentier, Y., Fardel, O., 2011. Drug transporter expression in human macrophages. *Fundam Clin Pharmacol* 25, 743-752.
- Morel, F., Beaune, P.H., Ratanasavanh, D., Flinois, J.P., Yang, C.S., Guengerich, F.P., Guillouzo, A., 1990. Expression of cytochrome P-450 enzymes in cultured human hepatocytes. *Eur J Biochem* 191, 437-444.
- Noel, G., Le Vee, M., Moreau, A., Stieger, B., Parmentier, Y., Fardel, O., 2013. Functional expression and regulation of drug transporters in monolayer- and sandwich-cultured mouse hepatocytes. *Eur J Pharm Sci* 49, 39-50.
- Pahlman, S., Ruusala, A.I., Abrahamsson, L., Mattsson, M.E., Esscher, T., 1984. Retinoic acid-induced differentiation of cultured human neuroblastoma cells: a comparison with phorbol ester-induced differentiation. *Cell Differ* 14, 135-144.

- Payen, L., Courtois, A., Campion, J.P., Guillouzo, A., Fardel, O., 2000. Characterization and inhibition by a wide range of xenobiotics of organic anion excretion by primary human hepatocytes. *Biochem Pharmacol* 60, 1967-1975.
- Pfeifer, N.D., Hardwick, R.N., Brouwer, K.L., 2014. Role of hepatic efflux transporters in regulating systemic and hepatocyte exposure to xenobiotics. *Annu Rev Pharmacol Toxicol* 54, 509-535.
- Prueksaritanont, T., Chu, X., Gibson, C., Cui, D., Yee, K.L., Ballard, J., Cabalu, T., Hochman, J., 2013. Drug-drug interaction studies: regulatory guidance and an industry perspective. *AAPS J* 15, 629-645.
- Qiu, X., Bi, Y.A., Balogh, L.M., Lai, Y., 2013. Absolute measurement of species differences in sodium taurocholate cotransporting polypeptide (NTCP/Ntcp) and its modulation in cultured hepatocytes. *J Pharm Sci* 102, 3252-3263.
- Ramboer, E., Vanhaecke, T., Rogiers, V., Vinken, M., 2013. Primary hepatocyte cultures as prominent in vitro tools to study hepatic drug transporters. *Drug Metab Rev* 45, 196-217.
- Richert, L., Tuschl, G., Abadie, C., Blanchard, N., Pekthong, D., Manton, G., Weber, J.C., Mueller, S.O., 2009. Use of mRNA expression to detect the induction of drug metabolising enzymes in rat and human hepatocytes. *Toxicol Appl Pharmacol* 235, 86-96.
- Schaefer, O., Ohtsuki, S., Kawakami, H., Inoue, T., Liehner, S., Saito, A., Sakamoto, A., Ishiguro, N., Matsumaru, T., Terasaki, T., Ebner, T., 2012. Absolute quantification and differential expression of drug transporters, cytochrome P450 enzymes, and UDP-glucuronosyltransferases in cultured primary human hepatocytes. *Drug Metab Dispos* 40, 93-103.
- Sharanek, A., Azzi, P.B., Al-Attrache, H., Savary, C.C., Humbert, L., Rainteau, D., Guguen-Guillouzo, C., Guillouzo, A., 2014. Different dose-dependent mechanisms are involved in early cyclosporine a-induced cholestatic effects in hepaRG cells. *Toxicol Sci* 141, 244-253.

- Stieger, B., 2011. The role of the sodium-taurocholate cotransporting polypeptide (NTCP) and of the bile salt export pump (BSEP) in physiology and pathophysiology of bile formation. *Handb Exp Pharmacol*, 205-259.
- Swift, B., Pfeifer, N.D., Brouwer, K.L., 2010. Sandwich-cultured hepatocytes: an in vitro model to evaluate hepatobiliary transporter-based drug interactions and hepatotoxicity. *Drug Metab Rev* 42, 446-471.
- Tchaparian, E.H., Houghton, J.S., Uyeda, C., Grillo, M.P., Jin, L., 2011. Effect of culture time on the basal expression levels of drug transporters in sandwich-cultured primary rat hepatocytes. *Drug Metab Dispos* 39, 2387-2394.
- Wilson, M.S., Graham, J.R., Ball, A.J., 2014. Multiparametric High Content Analysis for assessment of neurotoxicity in differentiated neuronal cell lines and human embryonic stem cell-derived neurons. *Neurotoxicology* 42, 33-48.

Legends to figures

Fig. 1. Sinusoidal drug transporter mRNA expression in monolayer-cultured human hepatocytes.

Relative expression of sinusoidal drug transporters was determined in primary human hepatocytes cultivated in a monolayer configuration (cPHH) and in freshly isolated primary human hepatocytes (fiPHH) by RT-qPCR, as described in Materials and Methods. Data are expressed in arbitrary units relatively to 18S RNA and are the means \pm SEM of at least 8 independent hepatocyte populations. Transporter expressions in cPHH expressed as % of those found in fiPHH (arbitrarily set at 100 %) are indicated by numbers in brackets. *, $p < 0.05$ (Student's t-test).

Fig. 2. Canalicular drug transporter mRNA expression in monolayer-cultured human hepatocytes.

Relative expression of canalicular drug transporters was determined in primary human hepatocytes cultivated in a monolayer configuration (cPHH) and in freshly isolated primary human hepatocytes (fiPHH) by RT-qPCR, as described in Materials and Methods. Data are expressed in arbitrary units relatively to 18S RNA and are the means \pm SEM of at least 8 independent hepatocyte populations. Transporter expressions in cPHH expressed as % of those found in fiPHH (arbitrarily set at 100 %) are indicated by numbers in brackets. *, $p < 0.05$ (Student's t-test).

Fig. 3. Drug transporter localization in monolayer-cultured human hepatocytes.

(A) Monolayer-cultured human hepatocytes morphology was examined by light phase contrast microscopy; bile canaliculi (BC), sinusoidal-like (SMb) and canalicular-like (CMb) membranes are indicated by black arrows. Bar = 15 μ M. (B) Immunolocalization of sinusoidal and canalicular drug transporters was performed as described in Materials and

Methods. Pictures correspond to single immunolabeling, knowing that membrane transporter-related fluorescence is green; blue fluorescence corresponds to 4,6-diamidino-2-phenylindole-stained nuclei. Data shown are representative of four independent experiments. Transporter-related membrane staining is indicated by white arrows. White stars indicate high background autofluorescence, most likely due to dead cells. Bar = 10 μ M.

Fig. 4. Sinusoidal drug influx activities in monolayer-cultured human hepatocytes.

Cultured human hepatocytes were incubated with the NTCP substrate taurocholate (43.4 nM), the OATP substrate E3S (3.4 nM) or the OCT1 substrate TEA (40 μ M) for 10 min, in the presence or absence of sodium (for taurocholate), 2 mM probenecid (for E3S) or 50 μ M verapamil (for TEA). Intracellular accumulations of substrates were then determined by scintillation counting. Data are the means \pm SEM of five (Taurocholate and TEA) or eight (E3S) independent experiments. *, $p < 0.05$ when compared to uptake in the presence of sodium (for taurocholate) or in the absence of inhibitors (for E3S and TEA) (Student's t-test).

Fig. 5. Canalicular drug transport activity in monolayer-cultured human hepatocytes.

Canalicular secretions of the BSEP substrate taurocholate, the MRP2 substrate CF and the MDR1/P-glycoprotein substrate rhodamine 123 were determined as described in Material and Methods. Data are the means \pm SEM of nine (Taurocholate), seven (CF) or four (Rhodamine 123) independent experiments, each being performed in triplicate. BEI values are indicated on the top of each graph. *, $p < 0.05$ (Student's t-test). FAU, fluorescence arbitrary unit.

Table 1. Categorization of drug transporter mRNA expression in freshly isolated primary human hepatocytes (fiPHH) and primary human hepatocytes cultivated in a monolayer configuration (cPHH).

Drug transporter	fiPHH	cPHH
NTCP	++	++
OATP1B1	++	++
OATP1B3	++	+
OATP2B1	++	++
OAT2	++	++
OCT1	+++	+++
MRP1	+/-	+/-
MRP2	++	++
MRP3	++	++
MRP4	+/-	+/-
MRP5	+/-	+/-
MRP6	++	+++
MDR1	+	+
BSEP	+	+
BCRP	+	+
MATE1	++	++

+++, very high expression (>100 units); ++, high expression (15-100 units); +, moderate expression (1-15 units); +/-, low expression (<1 unit).

Relative expression to 18S RNA

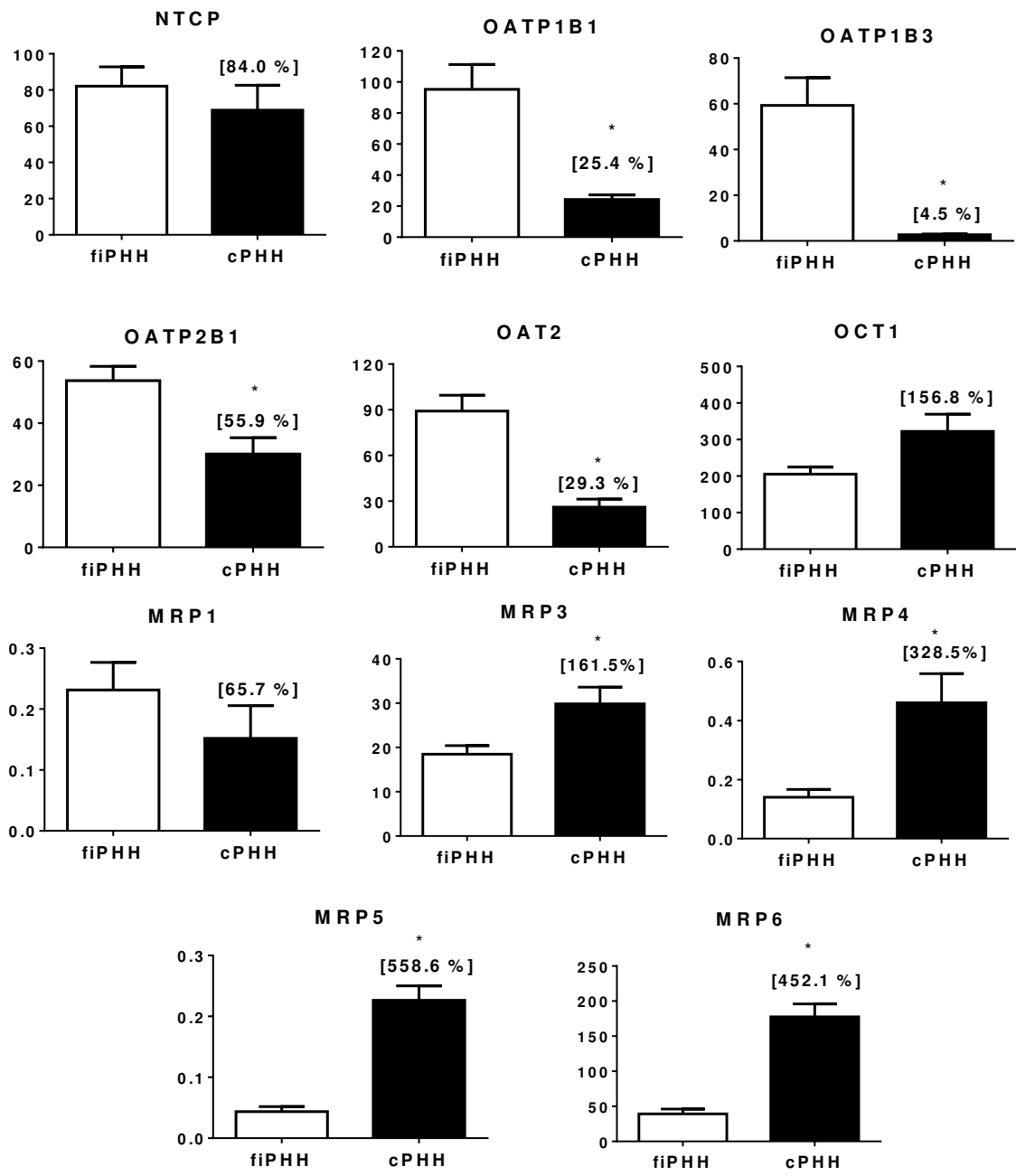


Fig. 1

Relative expression to 18S RNA

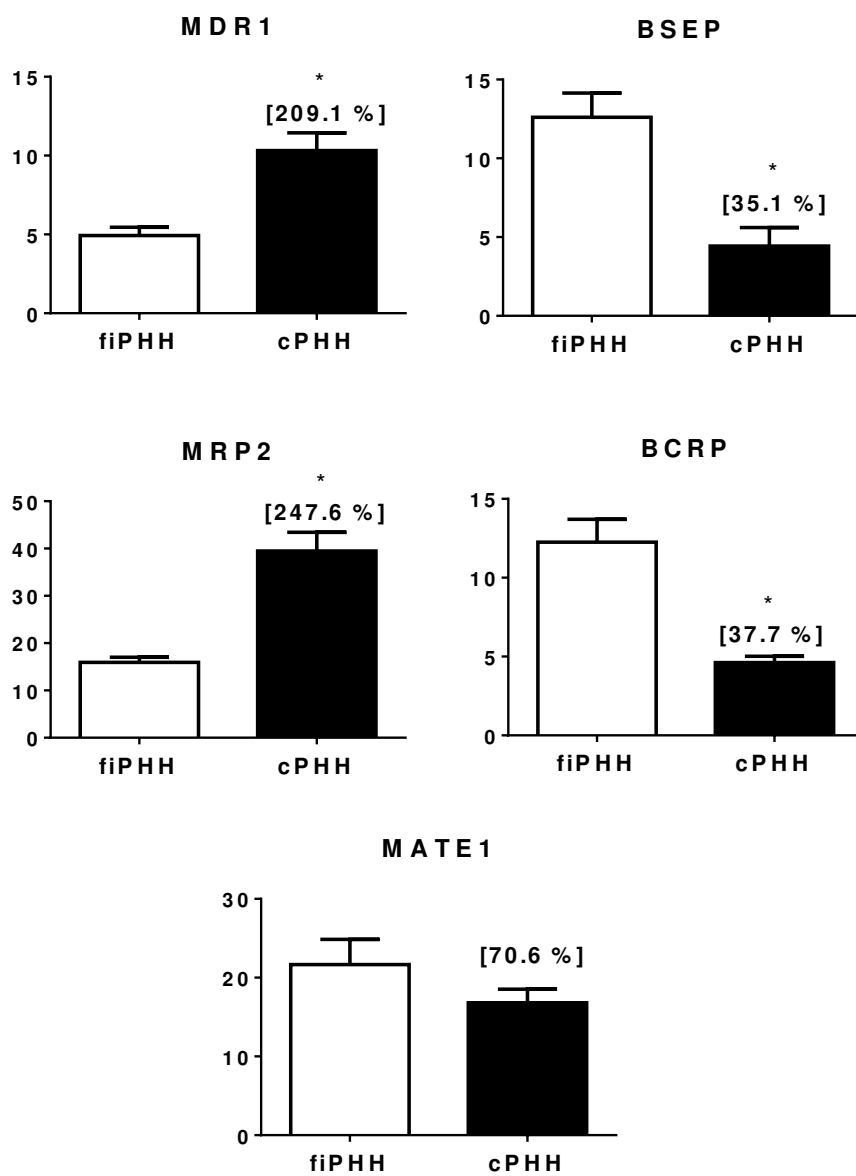
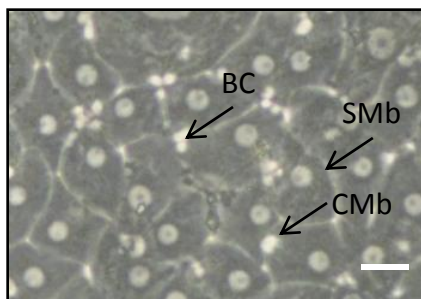


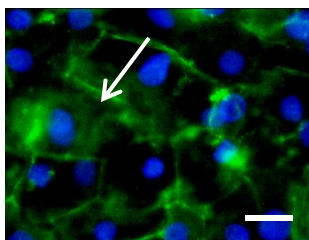
Fig. 2

A

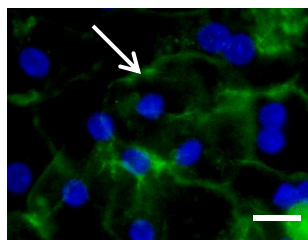


B

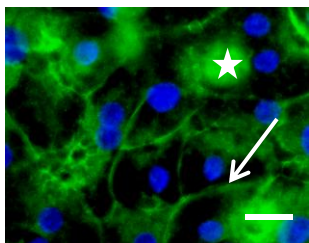
OATP1B1



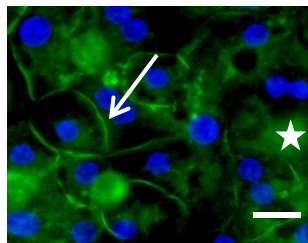
OATP2B1



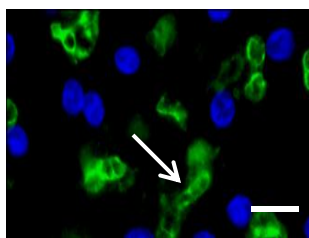
OCT1



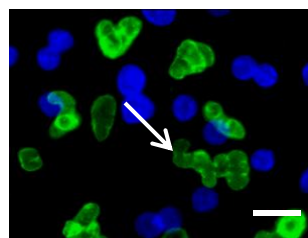
MRP3



P-glycoprotein



MRP2



Ig control

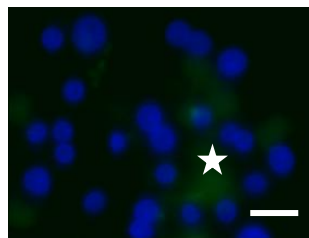


Fig. 3

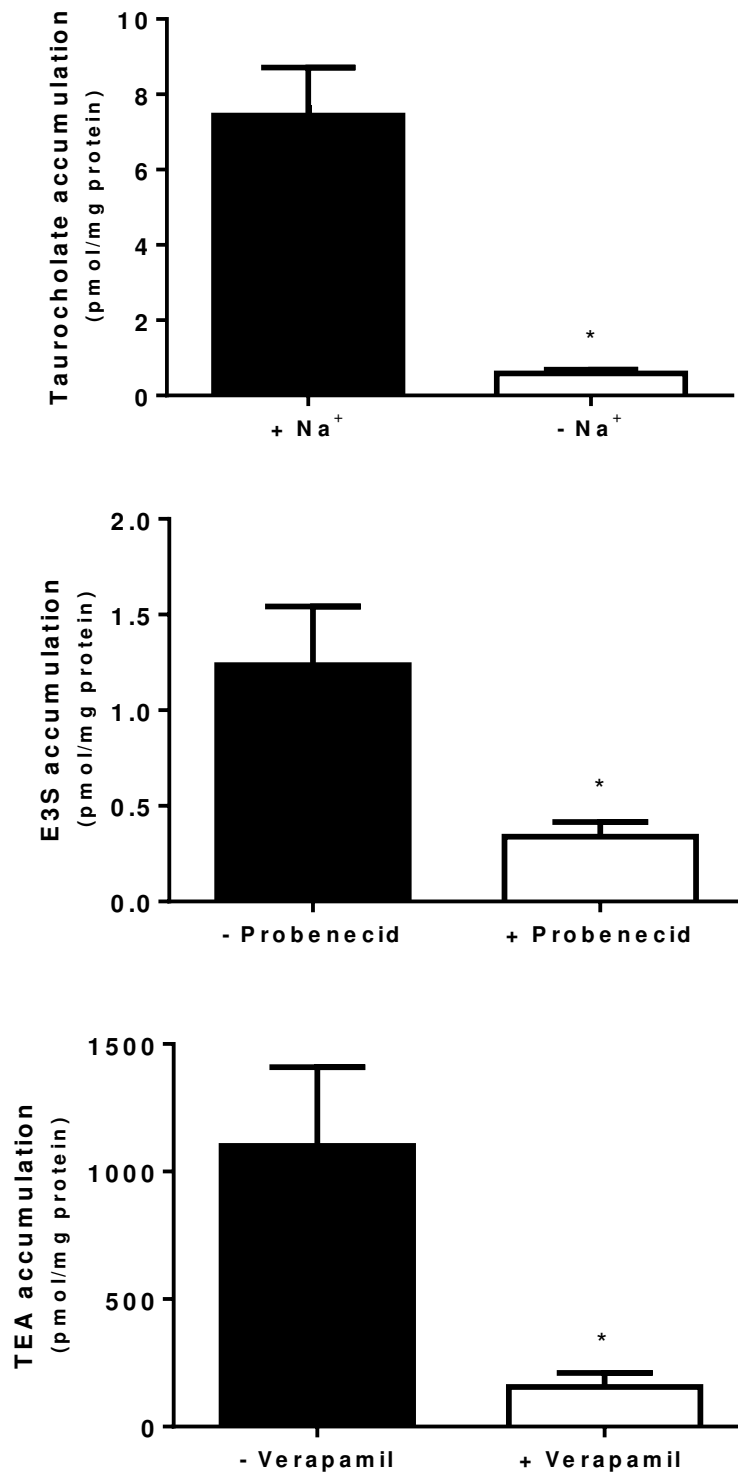


Fig. 4

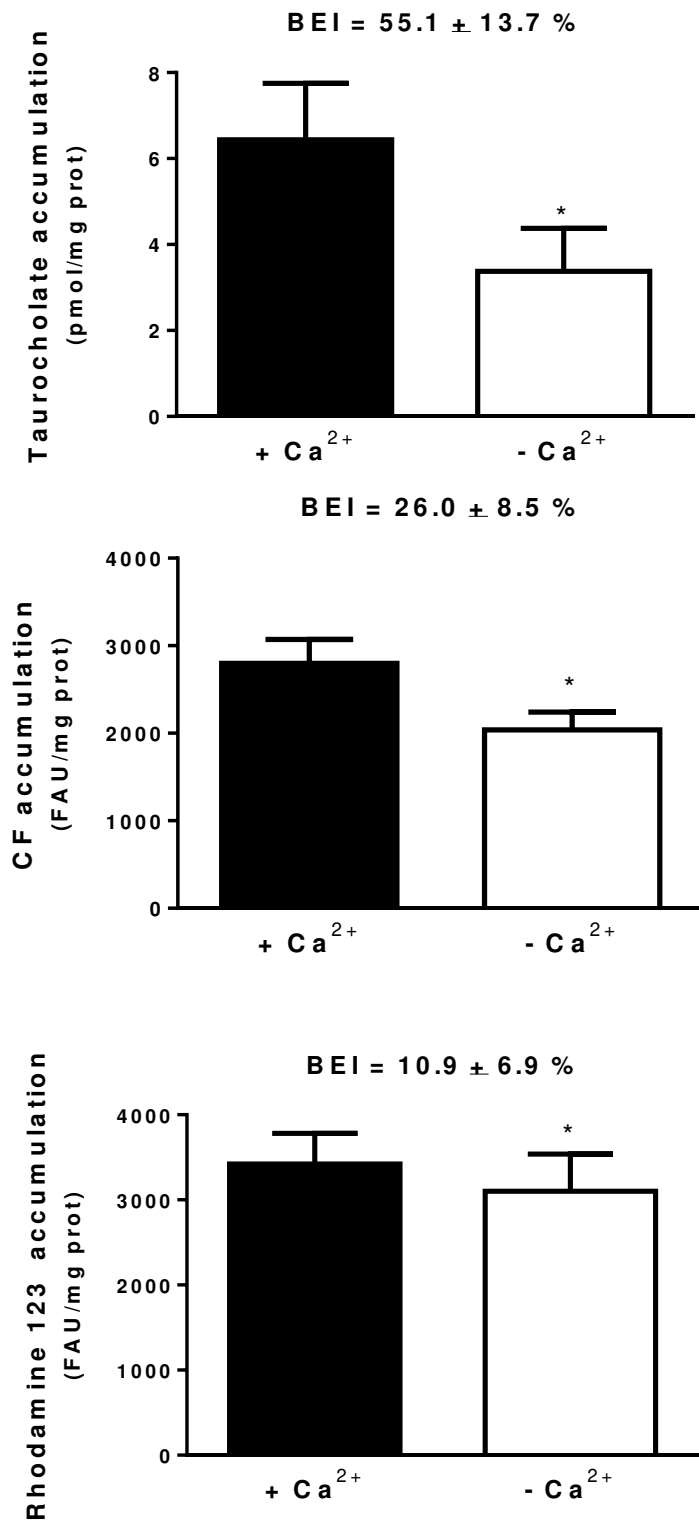


Fig. 5

Highlights

- Monolayer-cultured human hepatocytes retain notable drug transporter mRNA expression
- OATPs, OCT1 and MRP3 are detected at the sinusoidal pole of cultured hepatocytes
- P-glycoprotein and MRP2 are expressed at the canalicular pole of cultured hepatocytes
- Uptake transport is maintained in long-term monolayer-cultured human hepatocytes
- Canalicular secretion is maintained in 10 day monolayer-cultured human hepatocytes